A SIMPLE METHOD FOR THE PLATING OF ANAËROBIC ORGANISMS.

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PLATE XXV.

(*From the Bacteriological Laboratory of the College of Physicians and Surgeons.*)

It is not without hesitation that the writer ventures to add another to the already long list of procedures for the separation of anaërobic bacteria. He is encouraged, however, to describe the method given below by the hope that it may prove useful to other workers in the same field by its extreme simplicity and ease of application, and by its economy of time, in that it makes possible the use of the usual plating method in anaërobic studies.

The most satisfactory methods employed heretofore for the separation of anaërobes have depended almost without exception upon the use of a hydrogen generator. This, with its accessory solutions for the removal of impurities derived from the zinc (lead acetate and silver nitrate), is a formidable apparatus, difficult to keep in order and complicated in application. It is hardly necessary to emphasize to those who have worked with anaërobic organisms the desirability of eliminating the necessity for the use of hydrogen.

The most efficient of the methods of anaërobic separation without hydrogen is that of Roux, who used ordinary glass tubes pointed at the ends. The inoculated agar is drawn into these, the ends are sealed in a flame, and practically anaërobic conditions secured. When colonies have developed, the glass is cracked; or, even better, as the writer has found, the entire agar cylinder is pushed out into a sterile Petri dish with a glass rod of suitably small caliber. The method of Roux, while simple and efficient, presents a number of practical disadvantages. The mass of agar used is of necessity very small and unless, therefore, the

dilution of organisms has been extreme, the colonies will be very close together. Again, the convexity of the surface of the agar cylinder renders microscopical study of the colonies very unsatisfactory. The last but chief objection to this method is that there is a considerable absorption of oxygen by the agar during the time in which it is cooling sufficiently to permit its inoculation. While this, of course, is insufficient to prevent the growth of anaërobic organisms, it is enough to mar any conclusion that could be drawn as to the behavior of such organisms under absolutely oxygen-free environments.

The method to be described, the writer believes, besides being a facile one for separation, permits the assumption of absolute anaërobiosis. It is due entirely to the suggestion of Prof. Hiss, who proposed to the writer the simplification for plating purposes of the usual pyrogallic acid methods, that a successful method was devised.

The apparatus used consists of two circular glass dishes, fitting one into the other as do the halves of a Petri dish, and similar to these in every respect except that they are higher, and that a slightly greater space is left between their sides when they are placed together. The dishes should be about three-fourths to one inch in height, they need be of no particular diameter although those of about the same size as the usual Petri dishes are most convenient. The sole requirement necessary for successful plating is that the trough left between the two plates when put together shall not be too broad, a quarter of an inch being most favorable.

Into the smaller of these plates the inoculated agar is poured exactly as is done into a Petri dish in the ordinary anaërobic work. Prolonged boiling of the agar before plating is not essential. When the agar film has become sufficiently hard on the bottom of the smaller dish, the entire apparatus is inverted. The smaller dish is now lifted out of the larger, and placed, still inverted, over a moist surface—a towel or the wet surface of the table—to prevent contamination. Into the bottom of the larger dish, which now stands open, there is placed a quantity (one to two drachms) of dry pyrogallic acid. Into this, over the pyrogallic acid,

the smaller dish, still inverted, is then placed. A strong solution of sodium hydrate is poured into the space left between the sides of the two dishes, in quantity sufficient to fill the

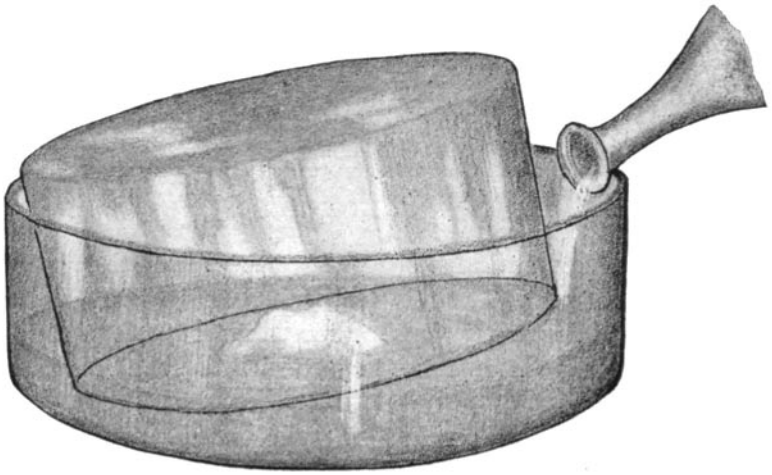

FIG. 1

FIG. 2

receiving one half full. While this is gradually dissolving the pyrogallic acid (and this is the only step which requires speed) alboline, or any other oil, is dropped from a pipette, previously

filled and placed in readiness, into the same space, thus completely sealing the chamber formed by the two dishes.

If these steps have been performed successfully, the pyrogallic solution will at this time appear of a light brown color, and the smaller plate, with its agar film, will float unsteadily above the other. Very rapidly, as the pyrogallic acid absorbs the free oxygen in the chamber,this plate is drawn down close to the other, and the acid assumes a darker hue, which remains without further deepening even after three or four days' incubation.

The accompanying photographs were made of cultures plated in this manner of B. tetani (Plate xxv, Fig. 1) and symptomatic anthrax (Fig. 2). The tetanus culture was separated in this way from a mixed culture containing two other organisms. The colonies thus grown can be studied and fished from in the same way as other colonies grown in Petri dishes.

While the observations the writer has made with this method are limited to the study of three strains of B. tetani and one of the bacillus of symptomatic anthrax, and in both cases to a series not exceeding a dozen platings in each, a few peculiarities of growth have been so constant in their occurrence in all the plates that they are thought worthy of record, especially in the light of the recent work of Smith in America and Tarozzi in Italy, which tends to show that complete anaërobiosis is not the principal factor for the cultivation of the so-called anaërobic organisms, but rather secondary to the presence of the proper nutriment.

In all of the plates observed, the growth has been slow, colonies of the smallest visible size rarely appearing earlier than twenty-four to thirty-six hours in the case of tetanus, or eighteen to twenty-four hours in the case of symptomatic anthrax. These colonies have very rapidly reached their maximum, which in the case of tetanus was rarely larger than about one to two millimeters in size, either in plain agar or in the 1 per cent. glucose agar used. While the initial inhibition in the growth may have been due to oxygen still present in the agar, yet the colonies have remained extremely compact and have even after five or six days' incubation failed to grow more profusely. This appears rather to support the points made by Smith and Tarozzi, since with com-

plete anaërobiosis and ordinary media a less luxurious growth was observed than that obtained by them with a less anaërobic environment but with more attention paid to the constitution of the media.

When fished and examined morphologically, after three or four days' incubation, many of the individuals of these colonies have, by the unevenness of their staining and variations of length, suggested degenerative change. In only two cases in the series observed were spores found in fishing from the colonies, and this in plates where, on account of slowness in the addition of the sealing covering of alboline, the pyrogallic solution was permitted to take on a deep black color (viz., saturation with oxygen). This latter point may in a measure account for the inability of the anaërobic bacteria to form spores under absolutely oxygen-free surroundings. And this again may tend to throw some light on the reason for the absence of the spores in some of the spore-forming anaërobic organisms in the circulation of infected subjects.

In conclusion, the writer takes pleasure in acknowledging his gratitude to Professor Philip Hanson Hiss, without whose suggestion these observations would hardly have been made.

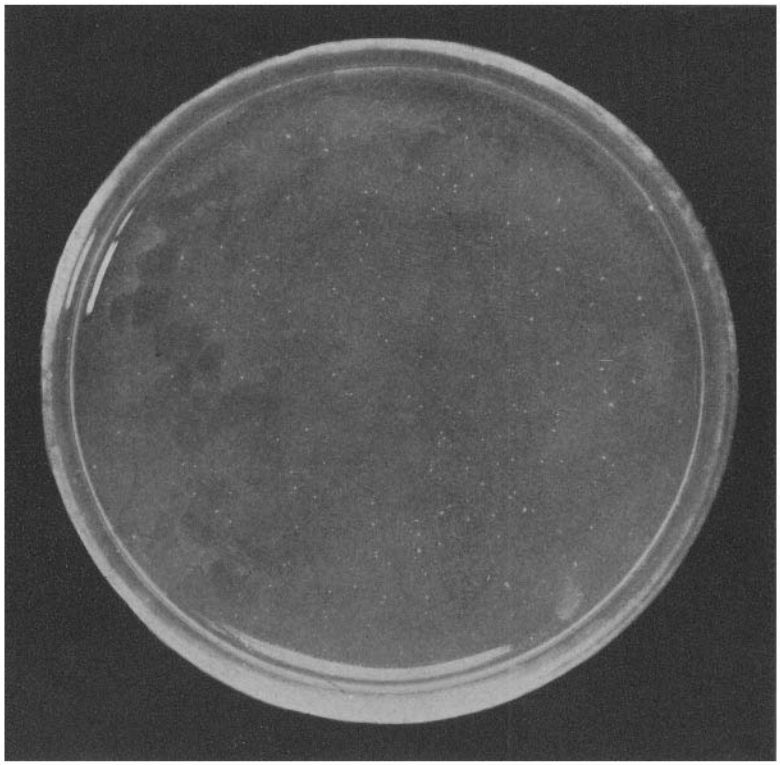

FIG. 1

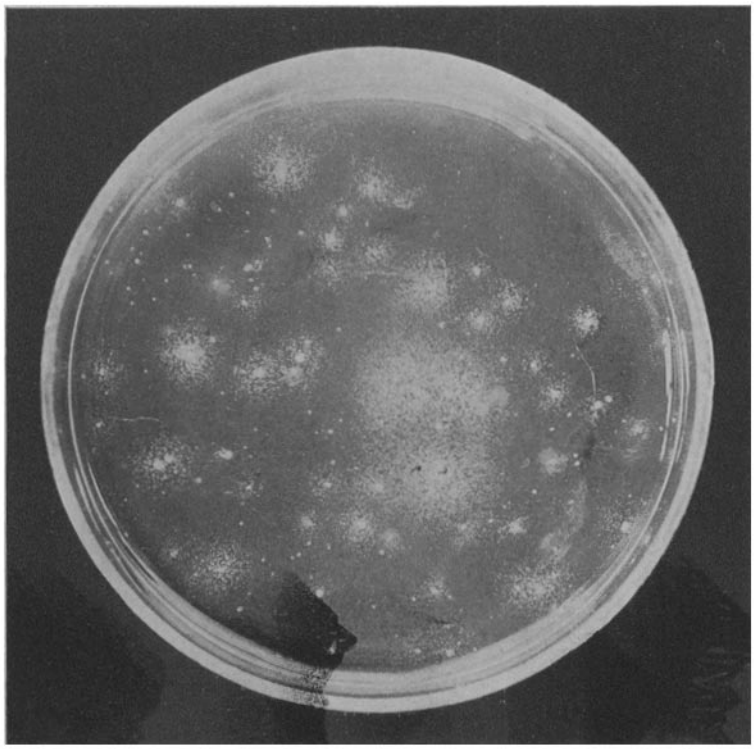

FIG. 2